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Arch Basic Clin Res 2023; 5(1): 208-217 • DOI: 10.5152/ABCR.2023.221323

Original Article

Serum Amino Acid Levels in Children with Autism Spectrum Disorder by Liquid Chromatography-Mass Spectrometry/Mass Spectrometry

Otizm Spektrum Bozukluğu Olan Çocukların Serum Amino Asit Düzeylerinin LC-MS/MS Metoduyla Belirlenmesi

Mehmet Ali Gül[1], Fatma Zuhal Umudum[2], Nezahat Kurt[3], Fatma Betül Özgeriş[4], Özge Nur Türkeri[5], Bahadır Turan[6], Onur Burak Dursun[7], Mevlüt Sait Keleş[8]

[1]Department of Medical Biochemistry, Amasya University, Faculty of Medicine, Amasya, Türkiye
[2]Department of Medical Biochemistry, Atatürk University, Faculty of Medicine, Erzurum, Türkiye
[3]Department of Medical Biochemistry, Erzincan Binali Yildirim University, Faculty of Medicine, Erzincan, Türkiye
[4]Department of Nutrition and Dietetic, Atatürk University, Faculty of Health Sciences, Erzurum, Türkiye
[5]Department of Nutrition and Dietetic, Lefke Europe University, Faculty of Health Sciences, Lefke, KKTC
[6]Department of Child and Adolescent Psychiatry, Karadeniz Technical University, Trabzon, Türkiye
[7]Departman of Child and Adolescent Mental Health, Kanuni Training and Research Hospital, Trabzon, Türkiye
[8]Department of Medical Biochemistry, Üsküdar University, Faculty of Medicine, İstanbul, Türkiye

Cite this article as: Gül MA, Umudum FZ, Kurt N,et al. Serum amino acid levels in children with autism spectrum disorder by liquid chromatography-mass spectrometry/mass spectrometry. *Arch Basic Clin Res.*, 2023;5(1):208-217.

ORCID IDs of the authors: M.A.G. 0000-0002-5849-0116, F.Z.U. 0000-0003-2502-9873, N.K. 0000-0002-1685-5332, F.B.O. 0000-0002-4568-5782, O.N.T. 0000-0001-8791-5331, B.T. 0000-0003-1190-9589, O.B.D. 0000-0003-2990-9851, M.S.K. 0000-0002-4905-219X.

ABSTRACT

Objective: Autism spectrum disorder is defined by repetitive and restricted behaviors and lack of social interactions and communication. It is described as a neurodevelopmental disorder that appears early in life. Autism spectrum disorder was later noted as multifactorial disorder caused by environment, genetic, and other related factors. Amino acids are the precursors of neurotransmitters, and they are involved in the pathogenesis of several diseases and have many functions and effects in the body. They are indicators of protein metabolism and nutritional status. Because of its important properties and its potential relationship with autism, this study aimed to investigate serum amino acid levels in 20 healthy children and 25 children with autism spectrum disorder.

Methods: Serum amino acid levels were analyzed by liquid chromatography-mass spectrometry/mass spectrometry.

Results: Autism spectrum disorder group had a lower serum concentration of gamma-aminobutyric acid, aspartic acid, and beta-aminoisobutyric acid levels compared to the control group, while glutamic acid, tryptophan, alanine, glycine, proline, ornithine, histidine, and serotonin levels had statistically higher concentrations ($P < .05$). And also, serum levels of asparagine, arginine, methionine, serine, valine, tyrosine, threonine, lysine,

ÖZ

Amaç: Otizm spektrum bozukluğu, tekrarlayıcı ve kısıtlı davranışlar ve sosyal etkileşim ve iletişim eksikliği ile tanımlanır. Yaşamın erken dönemlerinde ortaya çıkan nörogelişimsel bir bozukluktur. Otizm spektrum bozukluğu daha sonra çevresel, genetik ve diğer ilgili faktörlerin neden olduğu çok faktörlü bir bozukluk olarak tanımlanmıştır. Amino asitler, nörotransmiterlerin öncüleridir ve birçok hastalığın patogenezinde yer alırlar ve vücutta birçok işlev ve etkiye sahiptirler. Protein metabolizmasının ve beslenme durumunun göstergeleridir. Önemli özellikleri ve otizm ile potansiyel ilişkisi nedeniyle, otizm spektrum bozukluğu olan çocukların serum amino asit düzeylerinin araştırılması amaçladı.

Yöntemler: Bu çalışmaya 20 sağlıklı çocuk ve 25 otizim spektrum bozukluğu olan çocuk dahil edilmiştir. Serum amino asit seviyeleri, sıvı kromatografi-kütle spektrometrisi/kütle spektrometrisi ile analiz edildi.

Bulgular: Otizm spektrum bozukluğu grubunda gama-aminobütirik asit, aspartik asit ve beta-aminoizobütirik asit düzeyleri kontrol grubuna göre daha düşükken, glutamik asit, triptofan, alanin, glisin, prolin, ornitin, histidin ve serotonin seviyeleri istatistiksel olarak daha yüksek konsantrasyonlara sahipti. ($p < .05$). Ayrıca asparagin, arjinin, metiyonin, serin, valin, tirozin, treonin, lizin, lösin, beta alanin, anserin, hidroksiprolin,

Sorumlu Yazar/Corresponding author: Mehmet Ali Gül, e-mail: mehmetali.gul@amasya.edu.tr

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Geliş Tarihi/Received: July 27, 2022
Kabul Tarihi/Accepted: November 2, 2022
Yayın Tarihi/Publication date: January 31, 2023

leucine, beta alanine, anserine, hydroxyproline, argininosuccinate, isoleucine, sarcosine, citrulline, histamine, phenylalanine, and glutamine were not found to be statistically lower or higher ($P > .05$).

Conclusion: The study indicated that when compared to the control group, children with autism spectrum disorder had statistically significantly decreased and increased variation in some serum levels of amino acid.

Keywords: Amino acid, autism spectrum disorder, LC-MS/MS

argininosüksinat, izolösin, sarkozin, sitrülin, histamin, fenilalanin ve glutamin serum düzeylerinde istatistiksel olarak farklılık bulunmadı ($P > ,05$).

Sonuç: Çalışma, sağlıklı kontrol grubu ile karşılaştırıldığında, otizm spektrum bozukluğu olan çocukların bazı serum amino asit düzeylerinin istatistiksel olarak anlamlı derecede azaldığını ve arttığını göstermiştir.

Anahtar Kelimeler: Amino asit, otizm spektrum bozukluğu, LC-MS/MS

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder and individuals with ASD typically have limited and repetitious behaviors and deficiencies in social interaction and communication.[1] Definition of autism has changed over time and autism was accepted under the roof of ASD with Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5).[2] The universality of ASD is increasing, with an estimated prevalence of 1.5% in developed countries.[3] Even if the cause of the increase is associated with increased awareness of the disease and modification in diagnostic criteria, increase in the incidence of ASD cannot be ignored.[4] Although several risk factors have been identified, there is no specific risk factor to explain this increased prevalence in the disease.[5]

In recent studies, some risk factors, neurobiological and genetics-based relationships, have been defined, but the causes of autism are not yet known, except that it is complex and multifactorial. Genetics has a significant role in the etiology of ASD, and research suggests that disease shows a high degree of genetic heterogeneity.[6]

Biomarkers are required to identify ASD, and amino acids are considered as potential biomarkers for ASD.[7,8] Among the tests used to investigate ASD, the importance of amino acid analysis is increasing and is expected to be among the priority tests.[8] Studies on amino acids have been conducted on individuals with ASD, but the limitations of the studies and the different results indicate that new studies are needed.[9]

Autism spectrum disorder studies have begun to be given more importance today due to reasons such as increasing incidence of the disease, not knowing the exact causes of the disease, and increasing social awareness. Amino acid analyses provide a lot of biochemical information. Neurological disorders seen in autism suggest impaired biochemical homeostasis. Amino acids and their derivatives have important roles in energy metabolism, detoxification, and neurotransmission processes. Different studies have shown that there may be a connection between amino acids and central brain functions and that amino acid changes or metabolism may play a role in the pathogenesis of various diseases.[10,11] Due to its important relationship with many diseases and metabolic pathways, we aimed to investigate the amino acid levels of children with autism and to compare them with healthy children.

MAIN POINTS

- Serum glutamic acid, tryptophan, alanine, glycine, proline, ornithine, histidine, and serotonin levels were increased in the autism spectrum disorder (ASD) group.
- Serum aspartic acid, beta-aminoisobutyric acid, and gamma-aminobutyric acid levels were decreased in the ASD group.
- There was no difference between the groups in serum levels of asparagine, arginine, methionine, serine, valine, tyrosine, threonine, lysine, leucine, beta-alanine, anserine, hydroxyproline, argininosuccinate, isoleucine, sarcosine, citrulline, histamine, phenylalanine, and glutamine.

METHODS

Ethics Committee

The study was approved by Clinical Research Ethics Committee of Atatürk University School of Medicine (No.B.30.2.ATA.0.01.00/141, decision/protocol number 12/7, date: December 08, 2016)

Patient and Control Group

This study included 25 children diagnosed with autism in adherence with the criteria described in the Diagnostic and Statistical Manual of Mental Disorders (fifth edition) and 20 healthy control children (control group) similar to the weight, height, and age distribution of the groups.

All subjects for both groups were selected from those living in eastern Anatolia of Türkiye and recruited from the Atatürk University Hospital. Study group subjects were diagnosed by clinicians specialized in the diagnosis and management of ASD, from Atatürk University Hospital Department of Child and Adolescent Psychiatry. For groups, exclusion criteria included history of neurological diseases (tuberous sclerosis, etc.), metabolic diseases (phenylketonuria, etc.), and autoimmune diseases.

A total of 45 children were enrolled in the study. Two groups of children were recruited, namely, the autism ASD group (n=25) and the healthy children (HC) group (n=20). Participants who failed to complete the biochemical analysis fully or accurately were excluded from the study. The volunteered children with ASD and their parents were referred to the Department of Child and Adolescent Psychiatry of Atatürk University Faculty of Medicine. The diagnosis and evaluation of ASD were performed by an experienced child psychiatrist according to the criteria of the DSM-5 and children's family members were asked to complete the autism behavior checklist (ABC). All children diagnosed with ASD were also assessed by the Disability Boards of the University and State hospitals (including child neurologist, child psychiatrist, and 7 other members) for state-funded access to learning and rehabilitation centers. Primary eligibility criteria for the ASD group participants were being ages of under 18, not having any known neurological disease, and not using psychiatric medication.

Biochemical Analysis

Venous blood of 5 mL was taken in the morning after 8 hours of fasting. Blood samples were collected into gel biochemistry tubes without anticoagulant. It was slowly overturned. After waiting for 30 minutes, it was centrifuged at 2000 rpm. Carefully separated serum was aliquoted into pre-prepared and numbered Eppendorf tubes. It was stored in −80°C deep freezer until the working day. The day before the working day, it was placed in the refrigerator at +4°C. Before starting the study, it was removed from the refrigerator and brought to room temperature.

Sample Preparation

Around 100 µL of serum sample (or calibrators and controls) was added into sample preparation tube. Then 100 µL of reagent 1 was added and vortexed for 10 seconds. After vortex, 200 µL of reagent 2 was added and vortexed for 10 seconds. After this step, 200 µL of reagent 3 was added and vortexed for 10 seconds. After vortexing, it was kept for 2 minutes and then vortexed again for 10 seconds. After 1 minute, 200 µL of reagent 4 was added and vortexed for 10 seconds and waited 1 minute after mixing. It was centrifuged at 5000 rpm for 3 minutes and 100 µL supernatant was taken into autosampler bottle. Then, 3 µL of the sample was injected into the LC-MS/MS system.

Liquid Chromatography-Mass Spectrometry/Mass Spectrometry Analyses

Amino acid analyses were performed in liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS) Tandem Gold Zivak LC-MS/MS. For serum amino acid analysis, Zivak free amino acid LC-MS/MS detection kit (ZV-3002-02C Amino acid biological fluids kit) was used in Zivak Tandem Gold LC-MS/MS system, following the kit instructions.

Statistical Analysis

Statistical analysis of the data was carried out using the Statistical Package for the Social Sciences version 20.0. (IBM SPSS Corp.; Armonk, NY, USA). Whether the data were normally distributed was evaluated using the Kolmogorov–Smirnov test. Student's *t* test, one of the parametric tests, was used for the data conforming to the normal distribution, and the Mann–Whitney *U* test, one of the nonparametric tests, was used for the data that did not comply with the normal distribution. The results were given as mean ± standard deviation (mean ± SD) and *P* values below .001 were contemplated very significant and *P* values below .05 were contemplated statistically significant. Information on some parameters was shown in the form of a box plot graph.

RESULTS

Demographic data of all participants are presented in Table 1. According to these data, there was no statistical difference between the control group and the ASD group in terms of age, weight, height, and body mass index. There were 2 girls and 23 boys in the ASD group, and

Table 1. LC-MS/MS System Parameters

Related Part	Description
Device	Zivak Tandem Gold LC-MS/MS System
Colon	Zivak-AA Colomn
Injection volume	3 µL
Gradient	00.00 minute 38% B 12:00 minute 65% B 12:01 minute 95% B 14:00 minute 95% B 14:01 minute 38% B 20:00 minute 38% B
Flow	0.25 mL/min
Ionization mode	ESI positive
CID gas	2.2mTorr
API nebulizing gas	55 psi
Scan time	0.900 sn
SIM width	1.5 amu
Drying gas	35 psi
Drying gas temperature	350°C
Needle voltage	5000 V
Shield voltage	600 V
Capillary voltage	50 V
Detector	1500 V

LC-MS/MS, liquid chromatography-mass spectrometry/mass spectrometry.

6 girls and 14 boys in the control group, a total of 4 girls and 37 boys.

Serum alanine levels obtained as a result of the analyses performed in the current study were found to be statistically significantly higher in the ASD group (515.157 ± 143.966 nmol/mL) compared to the control group (371.848 ± 101.802 nmol/mL) ($P < .05$). Box plot graph of the results of alanine (ALA) and control groups is shown in Figure 1.

In the ASD group, glutamic acid (171.99 ± 76.410 nmol/mL) was statistically significantly higher than the control group (90.678 ± 27.867 nmol/mL) ($P < .001$).

In the ASD group, glycine levels (377.188 ± 110.551 nmol/mL) were statistically significantly higher than the control group (278.080 ± 54.64 nmol/mL) ($P < .001$). Box plot graph of glycine acid values in ASD and control groups is shown in Figure 2.

Histidine levels in the ASD group (98.257 ± 21.953 nmol/mL) were statistically significantly higher than the control group (61.084 ± 11.612 nmol/mL) ($P = .001$).

Ornithine levels (164.249 ± 61.424 nmol/mL) in the ASD group were statistically significantly higher than the control group (96.3356 ± 25.361 nmol/mL) ($P < .001$).

In the ASD group, proline levels (234.699 nmol/mL ± 63.352 nmol/mL) were statistically significantly higher than the control group (175.690 ± 48.667 nmol / mL) ($P = .001$).

Serotonin levels were found to be approximately 2 times high in children with ASD compared to the control group (10.790 ± 5.209, 5.3013 ± 3.914, respectively, $P < .000$).

In the ASD group, tryptophan levels (46.898 ± 8.490 nmol / mL) were statistically significantly higher than the control group (30.534 ± 7.241 nmol/mL) ($P = .001$).

Gamma-aminobutyric acid (GABA) levels in the ASD group (1.112 ± 1.126 nmol/mL) were statistically significantly lower than the control group (7.291 ± 5.468 nmol/mL) ($P < .001$).

Aspartic acid levels in the ASD group (22.220 ± 7.400 nmol/mL) were statistically significantly lower than the control group (38.475 ± 9.793 nmol/mL) ($P < .001$). Box plot graph of aspartic acid values in ASD and control groups is shown in Figure 3.

Beta-aminoisobutyric acid levels (2.535 ± 1.061 nmol/mL) in the ASD group were statistically significantly lower than the control group (3.583 ± 1.070 nmol/mL) ($P < .001$).

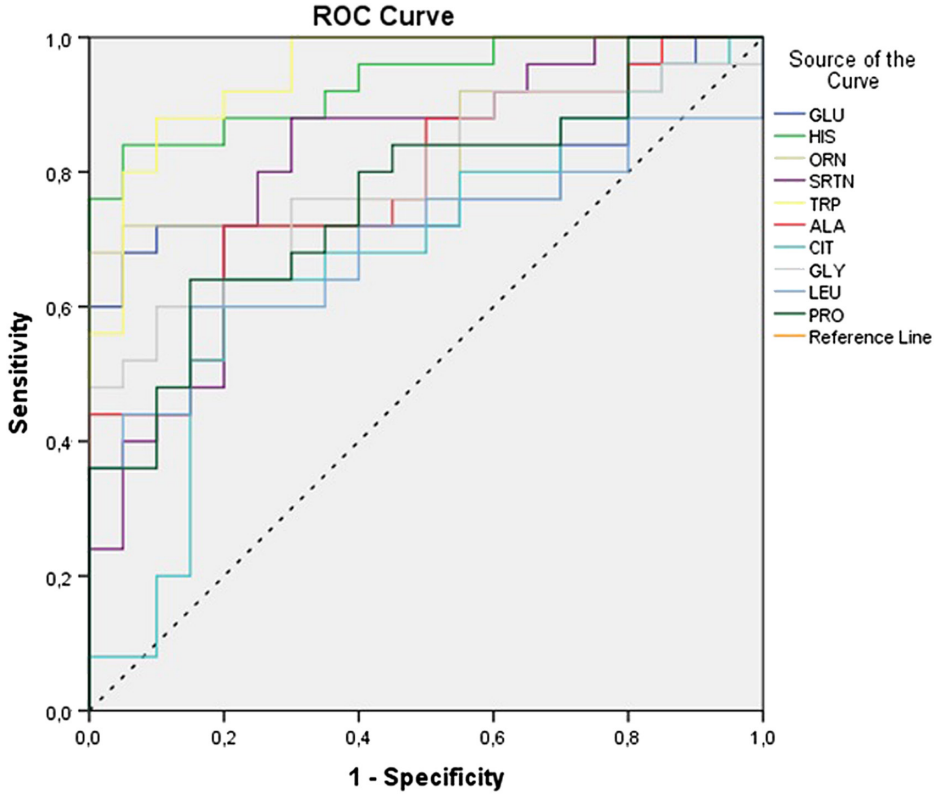


Figure 1. ROC analysis graph of amino acids with high serum levels in the ASD group. ASD, autism spectrum disorder; ROC, Receiver Operating Characteristic Curve.

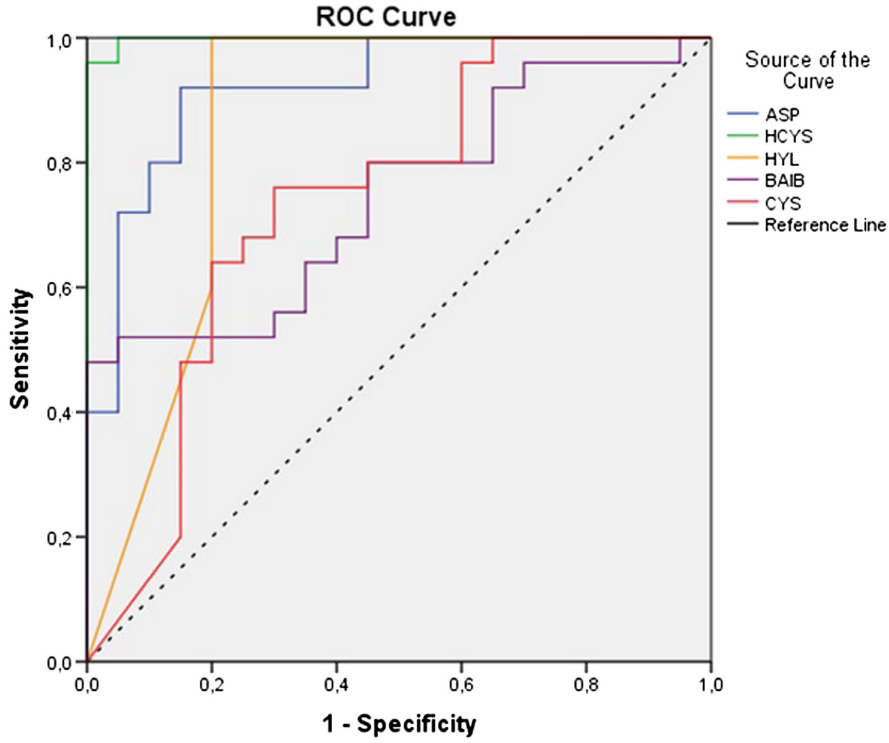


Figure 2. ROC analysis graph of amino acids with low serum levels in the ASD group. ASD, autism spectrum disorder.

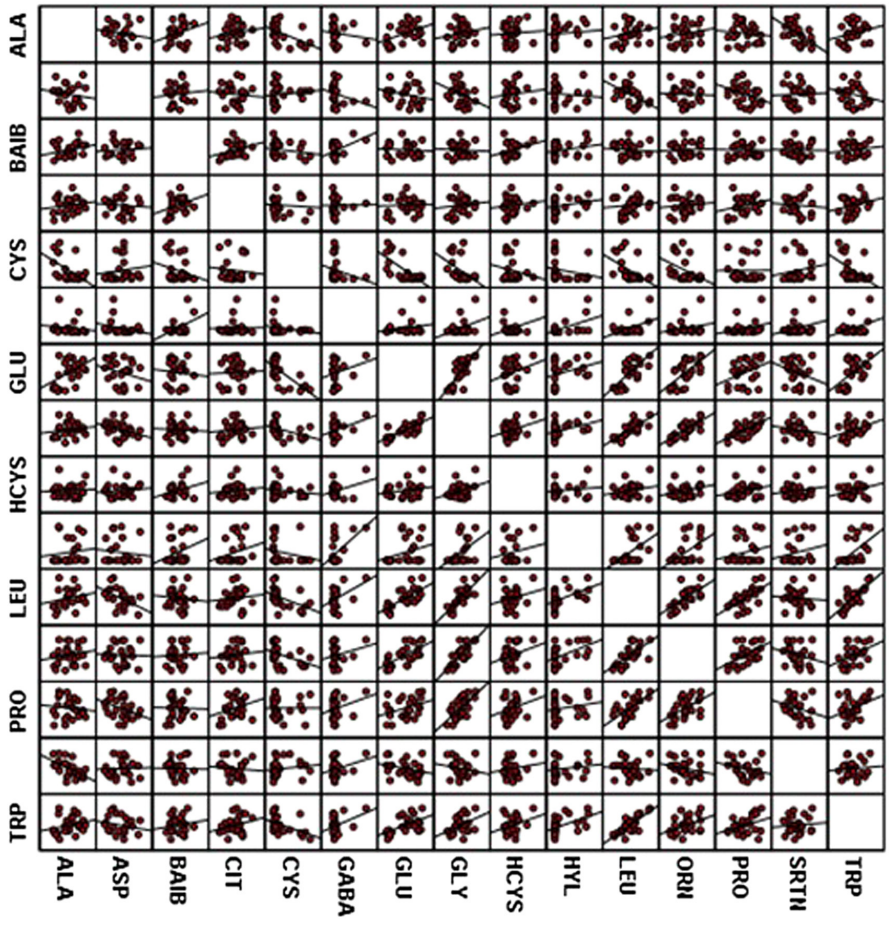


Figure 3. Person correlation matrix distribution plot of amino acids with different ASD levels. ASD, autism spectrum disorder.

Table 2. Demographic Information of the Groups.

	ASD Group (N=25)	HC Group (N=20)	
	Mean ± SD	Mean ± SD	*P*
Age (year)	9.08 ± 3.415	7.50 ± 3.086	.115
Gender (female/male)	23 / 2	14 / 6	-
Height (cm)	131.00 ± 15.365	123.250 ± 18.741	.135
Weight (kg)	31.160 ± 10.624	27.750 ± 10.764	.293
BMI	17.8 ± 3.7	17.6 ± 2.2	.813

ASD, autism spectrum disease; BMI, body mass index; HC, healthy control; SD, standard deviation.

No statistically significant difference was found in other amino acid levels. Amino acid levels measured in all groups are presented in Table 2.

DISCUSSION

In this study, we compared serum amino acid values in children with ASD and healthy children. Levels of serum Ala, Cit, Glu, Gly, Her, Leu, Orn, Pro, Srnt, and Trp were found to be significantly higher in the ASD group, while Asp, beta aminoisobutyric acid, and GABA levels were found to be low. There was no statistical difference in asparagine, arginine, methionine, serine, valine, tyrosine, threonine, lysine, leucine, beta-alanine, anserine, hydroxyproline, argininosuccinate, isoleucine, sarcosine, citrulline, histamine, phenylalanine, and glutamine in serum levels between the 2 groups.

Various risk factors for ASD have been identified, but none of them have been considered sufficient on their own; its etiology is generally defined as a genetic familiarity connected with an environmental effect.[12] There is a great need for biomarkers for the early identification of ASD and its causes, to improve and confirm the behavioral diagnosis.[7] Laboratory research on ASD is multifaceted and relies on a range of analytical platforms. Therefore, biomarker studies are expensive, challenging, and require highly advanced technical knowledge from researchers.[8] Investigation of the basis of the disease, molecularly and biochemically, is extremely important in understanding its pathophysiology and guiding clinical solutions.[13] The current research data assumed that certain amino acids are located in autism pathogenesis, but studies do not show exactly which amino acids are involved in the etiology of autism.[8] The importance of amino acid analysis in the tests used in the investigation of disease is increasing and is expected to become one of the primary tests.[8]

Glutamic acid is one of the most significant excitatory neurotransmitters in the central nervous system. It can easily cross the blood–brain barrier.[14] Glutamate is vital in memory, functions in learning, postsynaptic stimulation, and plays a role in taste and skin pain perception.[15] Anomalies in glutamatergic neurotransmission may be involved in the pathophysiology of autism.[16] Adams et al, in their study on plasma amino acids, showed that there was a significant increase in glutamate level in the group with ASD than the healthy group.[9] Although some studies have found different results, this may be due to the high age difference between the study groups. In a study conducted in Japan, serum glutamate levels were found to be significantly higher in adult patients with autism than in normal healthy controls.[16] In the study by Shinohe et al, it was discussed that high adult glutamate levels may occur at an earlier stage in childhood and this situation may be permanent. Therefore, it has been recommended to research glutamate levels in children with autism.[16] They also stated that measuring levels of serum glutamate in children without and with autism may be important in determining the role of glutamate as a serological marker in children. In this study, serum glutamate levels were found to be significantly higher in children with autism.

The balance between GABA and glutamate is crucial for brain function, and some psychiatric and neurological diseases may be associated with an imbalance between GABA and glutamate.[17] Body fluids reflect the physiology and pathology of the brain, and GABA and glutamate-dependent neurotransmission are important to understand the functional status. It has been reported that people with ASD have higher plasma GABA levels compared to the control group.[18,19] Unlike this study, we found that levels of serum GABA in children with ASD were significantly lower than in the healthy control group.

Shimmura et al suggest that plasma glutamine levels can be used as a diagnostic tool for early detection of autism.[20] Ghanizadeh suggested that a glutamine synthetase inhibitor may increase inflammation in patients with ASD.[21] In the studies by Bugajska et al[14] in serum and Shinohe et al[16] in plasma, no difference was found between glutamine levels of children with autism and healthy children. In our current study, in parallel, there was no difference in glutamine levels between the groups.

There are different results in the studies of alanine in the urine. In the study by Ming et al, alanine levels were found lower in the urine of children with autism compared to the control group. They stated that low alanine level in the urine of children with autism may be related to oxidative stress.[22] Contrary to this study, Mavel et al showed that they found high alanine levels in the urine samples of the autistic group.[23] While Ming et al[22] reported that beta-alanine levels were significantly decreased in the ASD group, Bala et al[24] found no difference between groups.

Table 3. Amino Acid Levels of ASD and Healthy Control Group.

Amino Acids	Amino Acids	ASD Group N = 25 Mean ± SD or Med(min-max) nmol/mL	HC Gruop N = 20 Mean ± SD or Med(min-max) nmol/mL	Direction	*P*
Alanine	Ala	515.1 ± 143.9	371.8 ± 101.8	↑	**.001***
Arginine	Arg	84.14 ± 29.31	75.01 ± 17.08	↑	.224
Argininosuccinate	Argsuc	0.34 ± 0.15	0.35 ± 0.23	↓	.942
Anserine	Ans	20.32 ± 7.82	17.08 ± 5.49	↑	.124
Asparagine	Asn	98.64 ± 15.83	95.41 ± 25.26	↑	.603
Aspartic Acid	Asp	22.22 ± 7.40	38.47 ± 9.79	↓	**.000***
Beta-aminoisobutyric acid	Baib	2.53 ± 1.06	3.58 ± 1.07	↓	**.002***
Beta-alanine	Bala	2.86 ± 1.07	2.45 ± 0.64	↑	.148
Cysteine	Cys	1.10 (0.00-4.60)	1.48 (0.00-3.4)	↓	**.007***
Citrulline	Cit	23.39 ± 6.19	19.65 ± 5.89	↑	**.046***
Gamma-aminobutyric acid	GABA	1.11 (0.51-5.10)	7.29 (0.00-20.1)	↓	**.000***
Glutamine	Gln	304.0 ± 64.8	322.7 ± 89.2	↓	.418
Glutamic acid	Glu	171.99± 76.4	90.6 ± 27.8	↑	**.000***
Glycine	Gly	377.18 ± 110.5	278.1 ± 54.6	↑	**.001***
Homocysteine	Hcyh	1.39 ± 0.72	8.42 ± 4.15		**.000***
Histidine	His	98.25 ± 21.95	61.08 ± 11.61	↑	**.000***
Histamine	Hista	0.016 (0.000-0.050)	0.017 (0.000-0.057)	↓	.722
Hydroxylysine	Hyl	0.247 ±0.038	0.534 ± 0.450		**.000***
Hydroxyproline	Hyp	24.51 ± 7.88	21.39 ± 8.26	↑	.203
Isoleucine	Ileu	69.84 ± 22.24	64.78 ± 13.86	↑	.379
Leucine	Leu	144.9 ± 47.6	120.3 ± 18.6	↑	**.035***
Lysine	Lys	152.12± 44.6	130.98 ± 32.81	↑	.084
Methionine	Met	33.17 ± 9.20	30.367 ± 5.88	↑	.244
Ornithine hydrochloride	Orn	164.24 ± 61.42	96.33 ± 25.36	↑	**.000***
Phenylalanine	Phe	100.48 ± 32.36	85.79 ± 18.49	↑	.078
Proline	Pro	234.6 ± 63.3	175.69± 48.6	↑	**.001***
Sarcosine	Sar	2.53 (0.00-7.20)	1.60 (0.00-3.80)	↑	.150
Serine	Ser	169.10 ± 39.39	154.55 ± 38.68	↑	.221
Serotonin	Srtn	10.79 ± 5.20	5.301 ± 3.91	↑	**.000***
Threonine	Thr	158.3 ± 42.6	150.4 ± 42.2	↑	.539
Trytophan	Trp	46.89 ± 8.49	30.53 ± 7.24	↑	**.000***
Tyrosine	Tyr	66.90 ± 16.78	59.44 ± 13.65	↑	.116
Valine	Val	213.54 ± 49.55	195.69 ± 39.35	↑	.196

ASD, autism spectrum disease; HC, healthy control; SD, standard deviation. *Statistically significant ($P < .05$).

Differences in study groups, ethnic origins, comparison methods, and study methods may be the reason for the differences in alanine levels.[8] On the other hand, in this study, we found high serum Ala levels in children with ASD, compared to the control group.

Glycine acts as both an inhibitory and an excitatory neurotransmitter.[8] It changes from an excitatory neurotransmitter at birth to act as an inhibitory neurotransmitter. If this does not occur at maturity, it can result in neurological disorders, including ASD.[25-27] Ming et al found that

Table 4. Data of ROC Analysis for Amino Acids

			95% CI	
	AUC	*P*	Lower Bound	Upper Bound
Glu	0.782	.001	0.640	0.924
His	0.934	.000	0.865	1.000
Orn	0.830	.000	0.708	0.952
Srtn	0.810	.000	0.681	0.939
Trp	0.948	.000	0.889	1.000
Ala	0.780	.001	0.646	.914
Cit	0.678	.042	0.516	0.840
Gly	0.786	.001	0.653	0.919
Leu	0.694	.027	0.537	.851
Pro	0.764	.003	0.626	0.902
Asp	0.922	.000	0.843	1.000
Hcys	0.998	.000	0.991	1.000
Hyl	0.860	.000	0.729	0.991
Baib	0.744	.005	0.601	0.887
Cys	0.737	.007	0.582	0.892

AUC, area under the curve.

glycine was significantly lower in the urine of children with autism compared to the control group, they stated that it was not clear whether there was a selective reduction in these amino acids from the proximal renal tubules.[22] Nadal-Desbarats et al found high urinary glycine levels in patients with autism.[28] In this study, serum Gly levels were found to be significantly higher in children with ASD compared to control group.

Bala et al found that histidine levels in plasma were higher in the autistic group compared to the control group.[24] Contrary to these data, Naushed et al showed that histidine levels were lower in the ASD group compared to the control group.[29] They stated that this situation may lead to gastrointestinal disturbances. High serum histidine levels have also been reported in healthy subjects. Similar to Bala et al, we found high serum His levels in children with ASD compared to healthy controls.

D'Eufemia et al found no difference between the serum proline levels of children with autism and the control group.[30] Tirouvanziam et al.[31] Chie Shimmura et al.[20] and Elbaz et al[32] found no significant difference in plasma proline levels in children with autism compared to healthy children. In this study, serum Pro levels were found to be higher in children with ASD.

Serotonin dysfunctions may contribute to some symptoms in some forms of ASD, such as social interaction disorders.[33] Compared to the healthy group, plasma tryptophan levels were reported to be decreased in ASD.[29] It has also been reported that high whole blood serotonin is found in more than 25% of children with ASD.[34] In our findings, serotonin was quite high in children with ASD. Also, Naushad et al[29] and Tu et al[35] reported low tryptophan levels in the fasting plasma of children with ASD. Contrary to these studies, in our study, serum Trp levels were observed to be higher in children with ASD.

D'eufemia et al[30] in serum and Elbaz et al.[32] Adam's et al.,[9] and Shimmura et al[20] in plasma found no significant difference in ornithine levels between the ASD and control groups. We, on the other hand, found that serum Orn levels were quite high in the group with ASD.

On platelets of children with autism, Rolf et al found lower levels of aspartic acid in the autistic group than in the control group.[36] Tirouvanziam et al found no difference in plasma aspartic acid levels between the autistic and healthy groups.[31] In our study, serum Asp levels were found to be lower in children with ASD.

Adam's et al[9] found higher beta-aminoisobutyric acid levels in plasma in the ASD group than in the control group. Contrary to these data, in this study, serum beta-aminoisobutyric levels were found to be statistically significantly lower in the ASD group compared to the control group.

Research on isoleucine and leucine, Tirouvanziam et al[31] in plasma, Perry et al in[37] cerebrospinal fluid, and Evans et al[38] in urine reported that isoleucine and leucine levels were lower in the ASD group. Contrary to these data, D'Eufemia et al[30] found higher in the ASD group in serum samples compared to the control group. The reason for these different findings may be due to different age, sample sizes ranges, and ethnicity of the subjects.[8] In this study, serum Leu levels were statistically higher in children with ASD. Q2

In the urea cycle, citrulline, an amino acid synthesized from ornithine and carbamoyl phosphate and also synthesized from arginine, is involved in inflammation and oxidative stress pathways.[8] In plasma, ElBaz et al.[32] Shimmura et al.[20] and Adams et al[9] in plasma and D'Eufemia et al[30] in serum found no statistically significant difference in citrulline levels of children with ASD compared to the control group. Contrary to these data, Tirouvanziam et al[31] reported that citrulline levels were significantly lower in the ASD group. In this study, serum Cit level was found to be statistically higher in children with ASD compared to the control group.

The histaminergic system has a critical role in sleep and some behaviors. Although not well researched in ASD,

the histaminergic system has been associated with many neurological diseases.[39] In 1988, Launay et al[40] reported that there was no difference between the autism group and the control group in their study on whole blood and urine samples. In our study, His levels in children with ASD were found to be quite high.

CONCLUSION

In this study, when the amino acid serum levels between the ASD and the control group were summarized, glutamic acid, tryptophan, alanine, glycine, proline, ornithine, histidine, and serotonin levels were statistically increased in the ASD group. Aspartic acid, beta-aminoisobutyric acid, and GABA levels were found to be statistically decreased in the ASD group. The detection of statistically different concentrations of certain amino acid levels in the ASD group compared to the healthy group suggests that these parameters may play a role in the disease and may also be helpful for laboratory finding in diagnosis.

Limitations

The small sample size is one of the limitations of the study.

Ethics Committee Approval: The study was approved with of the Atatürk University Medicine Faculty Clinical Research Ethical Board Commission. (Date: December 08, 2016, Decision No: B.30.2.ATA.0.01.00/141).

Informed Consent: Written informed consent was obtained from the families of the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A.G.; Design – M.A.G.; Supervision – F.Z.U., M.S.K; Resources – M.A.G.; Materials N.K., F.B.O., O.T.; Data Collection and/or Processing – B.T., O.B.D.; Analysis and/or Interpretation – N.K., F.B.O., O.T.; Literature Search – M.A.G., N.K.; Writing Manuscript – M.A.G., N.K.; Critical Review – F.Z.U., M.S.K.;

Declaration of Interests: The authors declare that they have no conflicts of interest.

Funding: This study was supported by the Atatürk University B.A.P. (No:2016/67).

Etik Komite Onayı: Bu çalışma için etik komite onayı Atatürk Üniversitesi'nden (Tarih: 8 Aralık 2016, Sayı: B.30.2.ATA.0.01.00/141) alınmıştır.

Hasta Onamı: Bu çalışmaya katılan hastaların ailelerinden yazılı bilgilendirilmiş onam alındı.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir– M.A.G.; Tasarım – M.A.G.; Denetleme – F.Z.U., M.S.K. ; Kaynaklar – M.A.G.; Malzemeler – N.K., F.B.O., O.T; Veri Toplanması ve/veya İşlemesi – B.T. O.B.D.; Analiz ve/veya Yorum – N.K., F.B.O., O.T; Literatür Taraması – M.A.G., N.K; Yazıyı Yazan – M.A.G., N.K. ; Eleştirel İnceleme – F.Z.U., M.S.K.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Bu çalışma Atatürk Üniversitesi B.A.P. (No:2016/67) tarafından desteklenmiştir.

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